REPRINT FROM

SOIL SCIENCE

RUTGERS COLLEGE

ASSIMILATION OF ORGANIC NITROGEN BY ZEA MAYS AND THE INFLUENCE OF BACILLUS SUBTILIS ON SUCH ASSIMILATION[1]

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan

By
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Olea

[1] Publication No. 156, Botanical Department, University of Michigan. Reprint from Soil Science, vol. 3, no. 2, February, 1917.

[Baltimore, 1917]

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STATEMENT OF PROBLEM

The aim of the work presented in this paper was first, to determine whether higher plants can utilize organic nitrogen directly without its being acted upon by microorganisms; second, to establish the relative importance of the compounds used; and third, to determine how the utilization of organic compounds by plants is affected by the action of a bacterium known to be able to decompose such compounds with the production of ammonia. The work embodies a series of experiments on the influence of different nitrogenous compounds, in sterile and inoculated cultures, upon the growth of seedlings of two varieties of Indian corn.

The problem was carried out under the direction of Professor J. B. Pollock of the Botany Department of the University of Michigan, and the author wishes here to make grateful acknowledgement to him for his assistance.

HISTORICAL INTRODUCTION

The discussion of soil fertility in modern times has centered upon the nitrogen problem. Nitrogen has long been known as one of the elements necessary for plant growth and is the one which must most continually be provided to keep up soil fertility, because it exists in such small quantities in the soil and is so easily removed by crops or by natural processes.

As long ago as 1835 Boussingault (5) showed that certain seeds contained as high as 4 to 7 per cent of nitrogen calculated on the dry weight basis. Later he (7) grew lupines, beans, and cresses in sand deprived of all nitrogen, and obtained about 1.3 per cent of nitrogen, showing probably a minimum requirement of that element in the plants. In the growth of soil fungi under nitrogen starvation conditions, Goddard (13) obtained from 1 to 2 per cent of nitrogen in the mycelium.

The growth of higher plants with an abundant supply of nitrogen shows that element to vary from 4.5 per cent in the leaves of red beets and in peas, 2.3 per cent in wheat grains, to 0.3 per cent in rye straw, ac-

[1] The data presented in this paper are from a thesis prepared in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

Received for publication October 25, 1916.

cording to Jost (17). With soil fungi grown in a rich nitrogenous medium, Goddard (13) found about 5 per cent in the mycelium. The analyses of different species of mushrooms, as given by Atkinson (1), shows the amount of nitrogen to vary from 2 to 6 per cent.

The plant has three possible sources of nitrogen, namely, free nitrogen of the air and inorganic and organic compounds in the soil. The nitrogen problem has centered around first one and then another of these sources, and in later times about the action of bacteria in relation to all three sources.

The view was held from the time of Aristotle to about the end of the eighteenth century that humus was the source of all nourishment of plants, though the early Romans knew that the growing of leguminous crops on the fields in some way increased their fertility and they applied this knowledge to their argriculture. Following the discovery of the chemical elements the relation of these elements to the nutrition of plants became the subject of numerous investigations.

The view of Aristotle dominated until about 1840 even though Ingenhouze thought plants were able to absorb free nitrogen from the air. At this time the great German chemist Liebig (26) concluded that plants absorb all or most of their nitrogen in the form of ammonium compounds, that the nitrogen problem was purely chemical, and that free nitrogen could not be utilized. He held firmly to these conclusions throughout his life. Liebig's opinion probably hindered further progress at this time, because he was recognized as one of the greatest chemists and his views were generally accepted.

A new view was established about 1860, namely, that nitric acid or nitrates furnish an excellent, if not the most available source of nitrogen for the great majority of plants. This was given by Boussingault (6, 8, 9) at the conclusion of experiments carried on from 1835 to 1860.

The problem of the *Leguminosae* increasing the nitrogen was not explained by these views of Liebig and Boussingault, and numerous experiments were carried out, among which were those of Lawes, Gilbert and Pugh (23). These early investigations finally culminated in the experiments of Hellriegel and Wilfarth (15). They showed in the clearest way that microorganisms present in the soil are the cause of the formation of the nodules upon the roots of leguminous plants, and that when these nodules are present the assimilation of free nitrogen occurs.

These conclusions in regard to bacterial action in the nitrogen problem were followed in a short time by further proof of the rôle of bacteria in nitrogen transformation. It was Winogradsky (56) in his bacteriological studies, who ultimately cleared up the physiology of the nitrobacteria, and his work has the right to be considered as one of the most important discoveries in plant physiology. He presented conclusive evidence of the existence of two kinds of nitro-bacteria; one of which pro-

duced nitrites from ammonia, and the other nitrates from nitrites. This discovery gave a clearer understanding of the old views of Liebig and Boussingault, and showed how organic compounds can become the source of nitrogen after first being ammonified and nitrified. In this process organic nitrogen is changed to inorganic which then is available for direct assimilation either as ammonium compounds or nitrates. Winogradsky (57, 58, 59) also discovered the non-symbiotic nitrogen-fixing bacteria living in the soil and studied their characteristics.

Organic Compounds

The direct assimilation of organic nitrogenous compounds was a part of the old humus theory and was held until Liebig's chemical theory began to prevail [Meyen (31) 1838]. Experiments tending to prove direct assimilation of such compounds were first made in 1857 by Cameron (10), with positive results. About 10 years later Wolf and Knop (60) also did similar work. Baessler (2), Lawes and Gilbert (22), and Berthelot (3) also have done some valuable work along this line. Since in these early experiments the significance of bacteria was not understood and the necessity for pure cultures was not recognized, all these early results are open to question.

Recent work on the assimilation of organic nitrogenous compounds has taken into account the possible action of bacteria and various investigations have indicated that these compounds are available for plants, although both negative and positive results have been obtained for the same compounds by various investigators. Strictly sterile conditions must be observed in testing accurately whether these compounds are directly assimilable or must first be acted upon by microorganisms to be ammonified or nitrified, or whether when so acted upon, they are rendered less toxic or more fully utilizable. There is also to be considered the difference in availability of the same substance for different plant species.

Suzuki (52) found that yellow lupines, potatoes, wheat and *Halesia hispidum* produced more asparagin from urea than from ammonium salts, while barley did not; and that, unlike nitrates, urea gave rise to asparagin in etiolated shoots.

Pryanishnikov and Lyebyedyev (40) in 1897 carried out experiments in sterilized and non-sterilized media with hippuric acid, urea, leucin, asparagin and aspartic acid. They found that none of the substances tested approached calcium nitrate as an effective source of nitrogen either in the sterilized or the non-sterilized media; also, that sterilization in all cases reduced the availability of the nitrogen of the organic substances, in most cases no gain being obtained in sterilized media.

Nakamura (37) in making quantitative comparison of asparagin and ammonium succinate as sources of nitrogen for barley, onions and *Asper-*

gillus oryzae, found that, in the case of the phanerogams, fully 50 per cent more growth was made where asparagin was added to the nutrient media than where the other compound was used. This was also true in the case of the fungus.

In 1898 Lutz (30) carried out some very extensive experiments upon the assimilation of organic nitrogen. These experiments were performed under sterile conditions, and thus fermentation products were excluded and nitrogen fixation prevented. The plants were grown in sterilized sand. The species used were, *Cucurbita maxima, Zea mays, Cucumis prophetarum, Helianthus annuus, Ipomaea purpurea, Onicus benedictus,* and *Cucumis melo.*

Trimeihylamin, dimethylamin, monomethylamin, diethylamin, propylamin and butylamin were all assimilated by the plants without first being fermented in the soil. Allylamin and benzylamin were found to be unfavorable to the growth of phanerogamic plants. The phenol-animes were toxic and the hydramines and pyridin bases were not assimilated. Tetramethylammonium and tetraethylammonium were not assimilated by phanerogamic plants. Among the alkaloids he found that caffin and quinin were toxic and cocain, atropin and morphin were not available.

Thompson (54) concludes from his studies with oats and barley that urea and uric acid have the same value for the grasses as nitric nitrogen, urea being slightly better than uric acid. His results indicate, however, that hippuric acid is detrimental to plant growth.

Pfeffer (39) has found that many heterotrophic organisms either require a supply of peptone or other proteins or attain their maximum development only when thus supplied. Phanerogams and algae can also employ as more or less valuable sources of nitrogen various organic substances such as: urea, glycocoll, asparagin, leucin, tyrosin, guanin, uric acid, acetamid, but none is as favorable to growth as sodium nitrate. He has also found that hippuric acid is decomposed by plants into glycocoll and benzoic acid, the latter of which is useless. He believed that the parts of the plant where such decomposition occurs are probably the same as those in which proteins are synthesized. Pfeffer holds that under natural conditions phanerogams rarely absorb organic nitrogenous compounds.

Schulze (49) quotes the investigations of a number of experimenters on the assimilation of leucin and tyrosin by plants and describes experiments of his own with lupines, vetches and castor beans, which showed that these chemicals could be used as sources of nitrogen by phanerogams.

Sawa (41), from investigations to determine if urea had any action on phanerogams, concluded that urea exercised an injurious action since the control plants made twice the growth of those in the solutions containing urea, and the branches were more vigorous on the control plants.

Kawakita (18) in his experiments on the effect of guanidin on plants found that solutions containing 0.5 gm. of guanidin in 250 c.c. killed

young barley plants in 3 days and that solutions one-fourth as concentrated killed the plants in 2 weeks.

Molliard (33) studied the value of asparagin and urea because, as he says, the assimilation of these two substances has been reported by others with different results. He grew his plant under sterile conditions and concludes from his experiments that these two substances maintain a nutrient rôle for higher plants.

Lefevre (24) in a series of experiments conducted with plants grown without carbon dioxide, found that glycocoll, alanin, tyrosin, and leucin not only furnish nitrogen, but also furnish the carbon required.

Schreiner and Reed (44) in their extensive studies tried guanin, although it is only slightly soluble in water. They used it in amounts varying from 1 to 40 parts per million, and in all of these concentrations it had a slightly beneficial effect upon the growth of wheat plants.

Guanidin carbonate, however, when tested on wheat plants in distilled water showed a very strong toxicity. When this solution was treated with carbon black, not only was the toxic action counteracted but the plants gave a better growth than the check in distilled water.

In a later publication (45) the same authors in their experiments found guanidin carbonate even in solutions so dilute as one part per million sufficient to kill wheat seedlings. Guanin was not harmful. Their experiments showed further that for wheat seedlings leucin and asparagin are not at all toxic. Alanin and glycocoll were slightly injurious at higher concentrations. Cumarin was extremely poisonous.

Bierema (4) reported that formamid and acetamid were not readily assimilated, although the latter was capable of supplying both nitrogen and carbon. Guanidin carbonate alone was not actively assimilated, but was somewhat more readily taken up in the pressence of calcium lactate, sucrose and glycerol. Uric acid was completely converted into ammonium carbonate, but less readily into urea. Leucin and tyrosin, especially the first, were readily assimilated, ammonium acetate more readily, especially in the presence of dextrose, and ammonium butyrate was still more readily assimilated.

Molliard (34) in further researches upon the utilization of organic nitrogen by higher plants, grouped his investigations under three main heads: (a) the action of various organic nitrogenous substances on the development and production of green and dry matter; (b) the total nitrogenous content of plants thus grown, and (c) the formation of protein substances from the absorbed nitrogen.

The following substances were used in the culture media in the ratio of 1:1000 parts: urate of sodium, aspartic acid, asparagin (1:500), glycocoll, legumin, cyanide of sodium, amygalin, hydrocyanic acid, leucin, tyrosin, myronate of potassium and alanin. Of these substances the first nine were utilized by the plants as shown by the increase in green and

dry matter over similar plants grown as checks. This utilization was the greatest in the case of urate of sodium, and decreased in order named down to leucin. Tyronsin, myronate of potassium and alanin were toxic to the roots only. The amount of protein nitrogen found in seedlings grown in the presence of asparagin and glycocoll was about twice the total nitrogen of the ungerminated seeds.

Hutchinson and Miller (16) in their work conclude that, while peptone and certain other nitrogenous compounds may be taken up and to some extent utilized by plants, they are unable to furnish the whole of the nitrogen required, or at any rate, to supply it with sufficient rapidity. They further conclude that their results are not sufficiently numerous to make it possible to trace any connection between the assimilability or non-assimilability of nitrogenous compounds and their constitution. They found it impossible to adhere to their original intention of sterilizing the media, for, although sterile media were most suitable, their employment was prevented by the impossibility of sterilizing many of the most desirable substances without more or less decomposition. They grouped the compounds experimented with under five heads, namely: (a) *readily assimilated*—ammonium salts, acetamid, urea, barbituric acid (with calcium carbonate), alloxan, humates; (b) *assimilated*—formamid, glycin, a. aminopropionic acid, guanidin hydrochloride, cyanuric acid, oxamid, sodium asparatate, peptone; (c) *doubtful*—trimethylamin (contrary to the results of Lutz), papa-urazine, hexamethylenetetramin; (d) *not assimilated*—ethyl nitrate, propionitrile, hydroxylamin hydrochloride, methyl carbamate; (e) *toxic*—tetranitromethane. This grouping, they affirm, is applicable only when peas are used and, as the authors suggest, it is possible that other plants may be able to utilize some of the substances which with peas have given negative results. Glycocoll in one culture gave an increase and in another a decrease.

Kossowicz (21) in his studies upon the assimilation of guanin and guanidin by mould fungi, found of about 10 fungi experimented with that all were able to utilize guanin as a nitrogen source, also guanidin under the conditions favoring the formation of ammonia. It is of interest that these results are different from those with the higher plants.

Schreiner (43) in his researches found that when creatin and nitrates are present less nitrates are used by the plant, although a larger plant growth takes place. The plant absorbs the creatin and builds it into its tissues. The author states that, upon his rather extensive investigations, he is ready to formulate the theory that the degeneration products of protein are absorbed directly by the plant from the soil and that the plant uses these units for building up the complex proteins as far as it is possible to do so. Since the plant must spend much energy in the building up of nitrates into amido groups of protein molecules, it is reasonable to suppose that the unit part of the complex molecule, when pre-

sented to the plant, will be used by it in preference to expending labor on the nitrate. The use of these decomposition products gives a different point of view to the problems of soil fertility.

Skinner (50) has shown in his experiments that the action of creatinin and creatin on growth is very similar. They had a beneficial effect on the growth where nitrate nitrogen was lacking and where only small amounts of nitrate were present, but when large amounts of nitrates were present these compounds produced no effect.

Skinner and Beattie (51) report that in all the plants experimented with asparagin is beneficial to growth, even when nitrate is present, although to a lesser degree.

Schreiner and Skinner (46) report upon some of the nitrogenous soil constituents as follows: Guanin at a concentration of 40 parts per million showed an increase in growth of 5 per cent over that of the growth in a distilled water control, and good root development. Asparagin showed, both in cultures with and without other sources of nitrogen, a decidedly beneficial effect upon the growth of plants. Guanidin produced a very decided toxic influence on growth. Glycocoll (amido-acetic acid) in water solutions was found to be beneficial. Alanin, in lower concentrations, was beneficial to growth, although in concentrations as high as 500 parts per million it slightly injured the roots of wheat seedlings.

Dachnowski and Gormley (12) in studies on bog plants and transpiration, together with the effect of glycocoll, state that the glycocoll is in part undoubtedly the gycocoll absorbed and assimilated.

Schreiner and Skinner (47) in experimenting upon the action of methyl glycocoll and glycocoll, found that the first was harmful, and the latter beneficial to the growth of plants.

It will be seen from the above review of the work on this subject that there is a great deal of contradiction in results obtained by different workers.

Bacterial Action

The process of nitrification was first shown in 1877 to be dependent upon the presence of certain micoorganisms, by Schloesing and Müntz (42). In 1893 Müntz and Coudon (36) showed for the first time that ammonia production in the soil is due to bacteria. However, in 1862 Pasteur (38) was the first to prove that the formation of ammonia from urea was brought about by the action of mocroorganisms. Within the last twenty years the work of numerous investigators shows that ammonia production from organic nitrogen is a function of most of the soil bacteria. Among the soil bacteria with this capacity is *Bacillus subtilis.*

Miquel (32) shows in his numerous experiments the effect of some of the species of bacilli which play an important rôle in the ammonifying of urea and splitting of uric acid into urea and other compounds. In

his conclusion he suggests that this splitting may play some part in the availability of these substances for the growth of plants.

Löhnis (29) found that soil bacteria rapidly convert urea into ammonium carbonate, probably by the action of *Urobacillus Pasteurii.*

The experiments regarding the decomposition of uric acid by bacteria, carried on by Liebert (25) showed that by aerobic bacteria the acid was broken up into carbon dioxide, ammonia, and the intermediate products, allantoin, urea, and oxalic acid.

Lipman (27) has recently determined that *B. subtilis* changes about 19 per cent of nitrogen present into ammonia.

Kelly (19, 20) has recently made extensive studies upon the biochemical decomposition of nitrogenous substances and ammonification, using commercial products such as casein, dried blood, cottonseed meal and linseed meal. The results showed that the different materials were converted into ammonia at greatly different rates and amounts.

New Experiments

In view of the contradictory results found by different investigators on the assimilation of organic nitrogen and in view of the desirability of testing more species of plants for their capacity of assimilating organic compounds, it was considered worth while to undertake the study of this problem with *Mays* plants grown with their roots in media free from bacteria except such as were intentionally inoculated into the cultures. The bacterium chosen was *B. subtilis.* This choice was so made because it is one of the common and widely distributed soil bacteria and has been shown to be capable of ammonifying organic compounds.

Methods and Techniqe

It is of the greatest importance that sterile, bacteria-free cultures be employed in investigations of soil bacteria, and especially in the case of experiments relative to the availability of organic nitrogen, for only thus can nitrification and ammonification be certainly prevented.

It is first necessary to select a medium which may be kept absolutely sterile throughout the experiment and which will permit the plants to make an active growth during a long period. Three media suggest themselves, namely, sand, water, and agar. Cultures in which each of these was employed were experimented with, and the latter was found to be the best adapted to the work at hand.

The plants which were placed in the sand cultures made a very poor growth and seemed to show evidence of a toxic influence. Warington (55) claims that such a material is in several respects a very unnatural medium for plant growth and is generally unsuited for this kind of investigation. Furthermore, it has so low a water-holding capacity that a culture when saturated contains about 60 per cent of inert material. Because of the small amount of water, some means of supplying sterile water

during the growth must be devised and this adds to the danger of contamininating the culture with fungi and bacteria.

Water cultures have been found satisfactory for a great deal of work by physiologists, but they require frequent change for the best results, and this is impractical under sterile conditions. Some means of aeration may be used. However, this can be done only at intervals, for continuous aeration is not practical with large numbers of cultures. Combes (11) suggests a method of aeration at intervals, but it requires special culture jars not easily obtainable. A preliminary experiment showed the poor growth of *Zea* plants in non-aerated water cultures.

Of the three media mentioned, the agar seemed then to afford the best substratum for the growth of the plants, the chemical compounds containing the mineral matters necessary for plant growth being added to it, of course. Some of the advantages of this media are: it makes possible the most rigidly pure cultures; the transparency of the agar permits the roots to be at all times visible; contaminations are easily recognized; and it affords a good mechanical support to the plants. The medium requires no attention beyond the initial preparation, that is, if a sufficient amount of medium is used at the beginning it does not require to be restored or renewed, even during a long period. This greatly lessens the danger of contamination. A 1-per cent agar solution was employed. This contained relatively little inert material, and sufficient water to last several months. The roots of the plants grew largely on the outside of the jelly-like, agar mass, which, as it gradually shrank away from the walls of the vessel, allowed good aeration of the roots. Agar was first employed as a culture substratum for green plants by Harrison and Barlow (14), who made use of it in their experiments with *leguminosae*. In the culture flasks in the experiment here recorded many of the plants grew well until all the water in the medium had been absorbed and the agar was dried down to a very small mass around the roots.

The agar medium was used in all of the experiments herein described, after the first preliminary ones. The roots of the plants were grown under sterile or inoculated conditions and the upper part exposed to the air. In order successfully to secure these conditions, some suitable culture jars had to be provided. For this purpose Erlenmeyer flasks of Jena, Resistenz or Bohemian glass of 700 and 1000-c.c. capacity were used. These were nearly filled with agar medium and sterilized in the autoclave for 20 minutes at 12 to 15 pounds pressure. Following the methods of Hutchinson and Miller (16), Schulow (48) and Combes (11) cotton plugs were placed in the mouths of the flasks, each plug rolled around a glass tube about 1 cm. in diameter and 15 cm. in length, through which the young plant could grow. This tube was also plugged with cotton. When the top was reached by the plant, the tube was withdrawn and the cotton pressed about the plant. This method allowed free growth of the leaves in the air, and aeration as well as sterile condition of the roots,

where this was desired. Each culture flask was wrapped with black paper to exclude light from the roots.

The experiments described here were carried out with two varieties of corn, namely, *Zea Mays everta,* Sturtevant (pop corn); and *Zea Mays indentata,* Sturt. (dent corn).

The pop corn seedlings were all grown in the greenhouse the first year, but the dent corn seedlings the second year were grown in the large south windows (9 feet high and 12 feet wide) of the laboratories of the Science Building, because the new botanical greenhouses were not completed and the old one was unavailable. The air of the rooms was kept moist by sprinkling the floors and having large pans of water exposed in the room. The cultures were maintained for two to three months.

Two methods were employed for measuring the growth of the plants. During the growth at various intervals and at the completion of the experiment the leaves were measured, and with the dent corn the dry weight of both the tops and the roots was obtained. The measurements were made first from the seed to the top of the youngest leaf and to this was added the length of each leaf from the stalk to its tip. The complete measurement of the plant was recorded. The measurements at intervals during growth did not reveal any special characteristics so they are not recorded in the data presented in this paper. The dry weights of the whole plant were determined after the removal of the remains of the seed. The roots were freed from the agar which remained about them by melting the agar in the autoclave and then washing the roots in boiling water. All of the substances occurring in the roots which are soluble in hot water were, of course, lost during this treatment. Each plant was then placed in a separate envelope and dried at a temperature of 80° C. to constant weight. These data of measurements and weights allow for accurate comparison with the checks which were grown in each series.

The results were recorded and studied in three different forms: by means of the tables compiled from the figures obtained and recorded later in this paper; by a comparison of photographs taken of the different sets grown at different times; and by graphs drawn for an easier and more ready comparison. Inoculated and sterile cultures were compared.

Twelve cultures were prepared in a set, each containing the same nitrogen source. Six of each set of 12 cultures were sterile and 6 were inoculated. One healthy seedling was planted in each flask. For the first series, the flasks were inoculated with 10 drops of soil water, prepared by shaking 5 gm. of soil with 50 c.c. of distilled water. In all of the other series where the flasks were inoculated, a pure culture of *B. subtilis* was used. A loop of bacteria was transferred with a sterile platinum wire loop from an agar slant to the warm liquid agar culture flask and thoroughly distributed through the medium by stirring with the sterile needle and shaking. In every case the inoculated flask showed a good growth of the bacteria.

Seed Sterilization

At the beginning of the work the seeds were sterilized by immersing in a water solution of mercuric chloride, 1:500, for 20 minutes. The seeds were first immersed in alcohol to remove any film of air. After the mercuric chloride treatment they were rinsed in sterile water to remove the sterilizing agent. This method was successful for the dent corn; but when the pop corn was so treated only a poor germination was obtained, and weak seedlings resulted from the few seeds that did germinate.

These results made it necessary to employ some other method for sterilizing pop corn, and following the suggestion of Lipman and Fowler (28), sulfuric acid (1.84 specific gravity) was tried. The best results were obtained by immersing the seeds for 4 minutes and then rinsing them in sterile water.

The sterilized seeds were placed on moist filter paper in sterile Petri dishes and allowed to germinate. In three or four days those that germinated well were transferred with sterile forceps to the surface of the agar medium in the flasks, and later the young shoots were directed into the glass tubes, which reached above the cotton stoppers. Germinating the seeds on agar was tried but the surface was too dry for the best results. Great care was used in selecting the seedlings to have them as nearly alike as possible, yet in spite of this precaution there was considerable difference in the rapidity of the growth during the first two weeks. Some that appeared healthy would not reach the tops of the tubes for a week or more after others which seemed equally as good. This difference is one of the greatest sources of error in the method used but its effect is minimized by the use of large numbers of plants.

Nutrient Solutions

The nutrient solution in these cultures was one which has been found to be most successful by Professor Pollock, after extensive experiments in his laboratory. The tribasic calcium phosphate was used instead of the acid phosphate to assure an alkaline medium. This has low solubility but by using an excess of the phosphate the solution was constantly kept supplied with a quantity sufficient for the growth of the plants. The amount of the different organic nitrogenous compounds to be used in the various solutions was determined upon the basis of furnishing in each solution the same amount of nitrogen that was present in the .004 M. solution of sodium nitrate used. Since peptone does not have a definite chemical formula, the nitrogen could not be accurately calculated, but it was estimated that 0.2 gm. of peptone in a liter of water would give the required amount of nitrogen.

A stock solution was used for the check and to this was added single nitrogenous substances in the preparation of the other media. The following is a list of the substances used in the stock solution, together with the

number of grams per liter of water of each substance used: calcium phosphate (tribasic) 1.240; magnesium sulfate 0.246; potassium chloride 0.298; ferric chloride 5 c.c. of .001 M. solution. This solution furnishes all of the elements necessary for the growth of green plants except nitrogen, and those obtained from water and carbon dioxide.

The other culture media were made up by adding each of the following substances to the stock solution (the number of grams of each used per liter of stock solution is indicated): sodium nitrate 0.340; urea 0.120; peptone 0.2; guanin 0.120; guanidin carbonate 0.180; benzamid 0.484; caffein 0.194; alanin 0.364; ammonium sulfate 0.264; asparagin 0.264; glycocoll 0.300; uric acid 0.168; diphenylamin 0.676; guanidin nitrate 0.122; hemoglobin 0.634; casein 0.459; linseed meal 1.120; cottonseed meal 1.090; malt 1.596; creatin 0.174.

With the exceptions of cottonseed meal, malt, peptone and linseed meal, all of the substances used in these nutrient solutions were chemically pure, and distilled water from the chemical laboratory was used in all cultures. The organic nitrogenous compounds employed were those prepared by C. A. F. Kahlbaum. The cottonseed meal and linseed meal were secured from a retail feed store, and the malt which was obtained from a brewery consisted of ground, sprouted barley grains.

Experiments

Pop Corn

Series I

The plants of this series were started the middle of October, 1914, and were harvested the middle of March, 1915. They were grown in the greenhouse in the following media: check consisting of the stock solution; and separate sets of media compound of stock solution to which sodium nitrate, urea, peptone, guanin and guanidin carbonate were respectively added. Half of the flasks were kept sterile while the other half were inoculated with soil water, as has been described above.

Soil water was not again used for inoculation because of bad results. The addition of this mixed culture of bacteria and fungi from the soil included some parasitic forms, which were detrimental to the plants. Therefore, in the succeeding series a pure culture of *B. subtilis* was used. The results of this series are incorporated in Tables I and II.

Series II

The cultures of Series II were started February, 1915, and harvested in June of the same year. They were grown in the greenhouse, and the same media were used as in Series I. The flasks which were here inoculated, had pure cultures of *B. subtilis* added, as has been described in the section on methods and technique. The plants of this series made a better and more uniform growth than those of Series I. Some of the plants

TABLE I

SERIES I, POP CORN, STERILE CULTURES
(October, 1914—March, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	4	255	187.50	100.0
		200		
		150		
		145		
Sodium Nitrate	4	425	342.50	182.6
		365		
		335		
		245		
Urea	3	300	251.70	134.2
		290		
		165		
Peptone	4	335	273.75	146.0
		315		
		225		
		220		
Guanin	2	180	147.50	78.6
		115		
Guanidin Carbonate*..				

* Plants small and died within a few days.

TABLE II

SERIES I, POP CORN, INOCULATED WITH SOIL WATER
(October, 1914—March, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	320	241.00	100.0
		275		
		250		
		190		
		170		
Sodium Nitrate	4	280	247.50	127.6
		260		
		230		
		220		
Urea	4	575	395.00	163.9
		375		
		330		
		300		
Peptone	4	230	211.25	87.6
		220		
		215		
		180		
Guanin	3	320	303.00	125.8
		290		
		280		
Guanidin Carbonate*..				

* Plants small and died within a few days.

showed contaminations which were parasitic. These were discarded from the data, and this was done in all of the following series. The contaminations in the flasks may have occurred on the seeds, some bacteria or fungi having survived the seed sterilization, or they may have gained entrance at the time of planting the seeds, when it was necessary to open the flasks. The results obtained here are similar to those of Series I, and may be studied by referring to Tables III and IV.

TABLE III

SERIES II, POP CORN, STERILE CULTURES

(February-June, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	12	290	235	100.0
		285		
		275		
		265		
		250		
		230		
		230		
		210		
		205		
		200		
		195		
		185		
Sodium Nitrate	7	450	384	163.4
		445		
		405		
		370		
		355		
		335		
		325		
Urea	8	370	299	127.2
		360		
		355		
		295		
		280		
		270		
		230		
		230		
Peptone	10	325	253	107.6
		305		
		290		
		275		
		250		
		230		
		230		
		200		
Guanin*				
Guanidin Carbonate*				

* Plants small, no roots, and soon died.

Series III

The plants in Series III were started the last of June, 1915, and harvested in about 8 weeks, the growth being very rapid during the long days and intense heat of the summer months. The greenhouse had rather poor means of ventilation and the glass was not painted so that the tem-

perature often reached 52° C., but the plants survived and made fairly good growth. The following nitrogen compounds were used in this set in addition to the check: sodium nitrate, urea, and peptone. The results are similar to those of the preceding series, and are recorded in Tables V and VI.

TABLE IV

SERIES II, POP CORN, INOCULATED WITH *B. SUBTILIS*

(February-June, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	11	365	246	100.0
		320		
		315		
		290		
		230		
		225		
		225		
		220		
		190		
		165		
		155		
Sodium Nitrate	7	610	407	165.4
		530		
		400		
		400		
		310		
		300		
		300		
Urea	11	400	370	150.4
		390		
		380		
		375		
		370		
		370		
		370		
		365		
		360		
		355		
		335		
Peptone	10	440	330	134.1
		420		
		360		
		320		
		315		
		315		
		310		
		305		
		265		
		250		
Guanin*				
Guanidin Carbonate*				

* Plants small, no roots, and soon died.

The study of the data upon these first three series revealed very similar results in all. A summary of these results is shown in Table VII and figure 1. Because of the large number of plants grown, some definite conclusions may be drawn from the experiments, which will be stated later.

Series IV

The plants of this series were grown at the same time as, and under similar conditions to those of Series III, except that water cultures were used instead of agar cultures. There was no provision made for aeration.

TABLE V
SERIES III, POP CORN, STERILE CULTURES
(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	290	210	100.0
		210		
		190		
		180		
		180		
Sodium Nitrate	3	320	283	134.7
		300		
		220		
Urea	3	180	178	84.7
		178		
		175		
Peptone	5	420	313	149.0
		370		
		300		
		245		
		230		

In addition to the check, media containing the following nitrogen compounds were used: sodium nitrate, urea, peptone, benzamid, caffein, alanin, ammonium sulfate, and asparagin. By consulting Tables VIII

TABLE VI
SERIES III, POP CORN, INOCULATED WITH *B. SUBTILIS*
(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	6	320	224	100.0
		260		
		250		
		230		
		195		
		190		
Sodium Nitrate	4	430	340	151.7
		410		
		310		
		170		
Urea	4	585	405	180.8
		430		
		325		
		280		
Peptone	2	340	300	133.9
		260		

and IX and comparing the growth with that in the agar medium, it can readily be seen that the growth in these water cultures was exceedingly poor in all cases, and not nearly equal to that in the agar cultures. The

TABLE VII

SUMMARY OF DATA OF LENGTHS OF LEAVES OF 110 POP CORN PLANTS GROWN IN DIFFERENT CULTURE MEDIA, UNDER STERILE CONDITIONS AND INOCULATED WITH *B. SUBTILIS*, 1914-1915

Culture	Sterile Cultures		Inoc. *B. subtilis*	
	Average length cm.	Per cent of average length of check	Average length cm.	Per cent of average length of check
Check	227.7	100.0	243.9	100.0
Sodium Nitrate	353.5	155.2	379.0	159.4
Urea	265.7	116.6	379.3	159.5
Peptone	272.3	119.6	325.0	133.2
Guanin*				
Guanidin Carbonate*				

* Toxic, no growth.

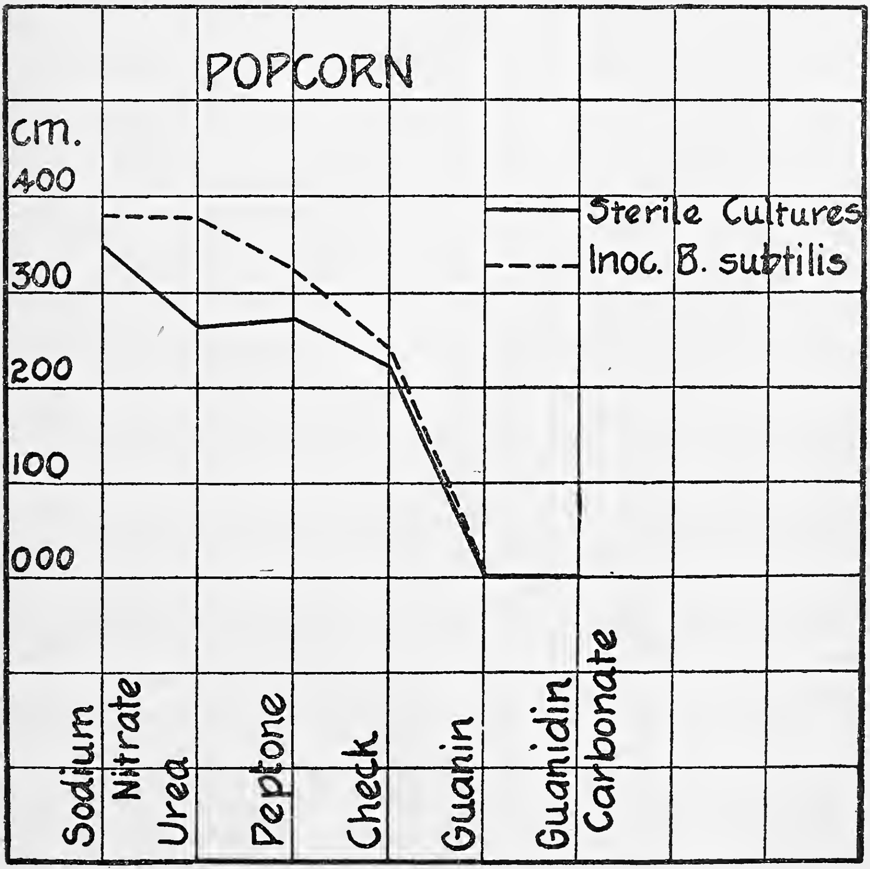

Fig. 1.—A summary of the results obtained from all of the pop corn plants grown in the experiment.

plants were weak and sickly. It was decided therefore, that unaerated water cultures were unsuitable for these experiments, and thereafter only agar cultures were used. The relative value of the nitrogenous substances in the water cultures was similar to that of these substances in the agar cultures, and may be used in connection with them.

TABLE VIII

SERIES IV, POP CORN, WATER CULTURES, STERILE CONDITIONS

(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	4	160	123.0	100.0
		120		
		110		
		100		
Sodium Nitrate	5	310	228.0	185.3
		240		
		240		
		190		
		160		
Urea	3	165	160.0	130.0
		160		
		155		
Peptone	4	230	170.0	138.0
		210		
		120		
		120		
Benzamid*				
Caffein	2	160	140.0	113.8
		120		
Alanin	4	140	127.5	103.6
		140		
		120		
		110		
Ammonium Sulfate	3	160	133.3	108.3
		120		
		120		
Asparagin	5	225	135.0	109.8
		120		
		115		
		110		
		110		

* Toxic, no growth.

Dent Corn

In the preceeding experiments some difficulty had been found in obtaining pop corn seedlings, and because of this fact and because it was advisable to try the effect of these substances upon another variety of the species, dent corn was used. Also other chemical substances were used as sources of nitrogen.

Series V

The plants of Series V were started in October, 1915, and harvested the following February. The plants were grown in the south window of one of the botanical laboratories. The light was not as good here as in

the greenhouse, but fairly uniform growth was obtained. The following nitrogen compounds were tested: sodium nitrate, urea, peptone, guanin, guanidin cabornate, guanidin nitrate, benzamid, caffein, alanin, ammonium sulfate, asparagin, glycocoll, uric acid, diphenylamine. In these, as in all the experiments, plants were grown in the stock solution as a check.

The results of these experiments are given in Tables X and XI. It is interesting to compare these results with those found in the growth of the pop corn plants. In general they are similar, but guanin which was toxic to pop corn was found to be quite beneficial to the dent corn seedlings.

TABLE IX

SERIES IV, POP CORN, WATER CULTURES, INOCULATED WITH *B. SUBTILIS*

(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	140	126	100.0
		135		
		135		
		120		
		100		
Sodium Nitrate	5	185	166	131.7
		175		
		170		
		170		
		150		
		150		
Urea	1	120	120	95.2
Peptone*				
Benzamid†				
Caffein	5	150	112	88.8
		115		
		110		
		95		
		90		
Alanin	2	130	120	95.2
		110		
Ammonium Sulfate	5	250	133	105.5
		160		
		90		
		85		
		80		
Asparagin	1	140	140	119.0

* No plants obtained. † Toxic, no growth.

Series VI

The plants of this series were started November, 1915, and the growth terminated during the next March. They were grown in a very poorly lighted window of one of the botanical laboratories, and consequently the growth was poor and very irregular. These facts must be considered in drawing any conclusion from the results of this series. The following nitrogenous substances were used: hemoglobin, casein, linseed meal, cottonseed meal, and malt. The results may be studied in Tables XII and XIII.

TABLE X

SERIES V, DENT CORN, STERILE CULTURES

(October, 1915—February, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	5	285	219	0.42	0.30	100.0
		250		0.28		
		230		0.30		
		180		0.25		
		150		0.25		
Sodium Nitrate	4	410	321	1.08	0.71	236.6
		325		0.62		
		280		0.63		
		270		0.52		
Urea	4	320	182	0.75	0.33	110.0
		180		0.30		
		160		0.22		
		110		0.06		
Peptone	4	345	296	1.18	0.66	220.0
		345		0.60		
		260		0.50		
		235		0.35		
Guanin	5	404	351	1.24	1.10	366.6
		385		1.40		
		365		1.10		
		350		1.30		
		250		0.46		
Guanidin Carbonate .	4	165	138	0.35	0.25	83.3
		155		0.30		
		120		0.15		
		105		0.20		
Benzamid*						
Caffein	6	150	109	0.20	0.19	63.3
		145		0.31		
		135		0.21		
		110		0.20		
		80		0.13		
		25		0.10		
Alanin	6	420	300	1.90	.94	313.3
		365		1.28		
		320		1.05		
		270		0.51		
		225		0.47		
		215		0.44		
Ammonium Sulfate...	3	300	275	0.65	.54	180.0
		290		0.62		
		235		0.35		
Asparagin	5	365	319	1.40	.84	290.0
		335		0.94		
		315		0.71		
		310		0.70		
		270		0.45		
Glycocoll	5	365	281	1.14	.63	210.0
		325		0.70		
		245		0.52		
		240		0.40		
		230		0.40		
Uric Acid	6	280	256	0.48	.44	146.6
		270		0.47		
		265		0.57		
		265		0.38		
		260		0.45		
		200		0.31		

TABLE X—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Diphenylamin*						
Guanidin Nitrate	4	160	134	0.31	.24	80.0
		135		0.27		
		130		0.22		
		110		0.15		

* No growth, died within three days.

Series VII

This was one of the most successful sets grown during the year. The plants were started in January, 1916, and harvested in about three months. They were grown in a well lighted window of one of the laboratories of the Science Building, and the room was well heated. The following media were used: distilled water, check, sodium nitrate, urea, peptone, guanin, alanin, ammonium sulfate, asparagin, uric acid, hemoglobin, casein, linseed meal, cottonseed meal, malt, and creatin.

The plants of this series all made good growth and some interesting results were obtained, which may be readily seen by a study of Tables XIV and XV. The results of this and of the other series are discussed later in this paper.

Series VIII

The plants of this series were started in February, 1916, and harvested about two months later. They were grown in large test tubes. These plants had only about half the amount of medium that the plants of other series had, and consequently the growth had to be terminated at an earlier stage, but nevertheless, interesting results were obtained. The plants were grown in the new botanical greenhouse under very ideal conditions of light and heat, and a very good and uniform growth resulted. The following substances were used: check, sodium nitrate, urea, uric acid, casein, and cottonseed meal. The detailed results of this series are given in Tables XVI and XVII.

Discussion

The data presented in this thesis, comprise observations upon 614 *Zea Mays* plants grown until the water supply became exhausted in one or more of the culture flasks of a series. The conclusions are based upon the results of growth of these plants. This number does not include those plants which showed extreme toxic effects when young and made no further growth, nor those discarded because they were attacked by fungi. The large number of the plants employed makes it possible for us to draw certain fairly definite conclusions from the data secured.

The percentage of possible error in such work is a large one and must be taken into account in interpreting the result obtained. There are sev-

TABLE XI

SERIES V, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(October, 1915—February, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	215	181	0.30	0.25	100.0
		200		0.28		
		190		0.30		
		175		0.30		
		170		0.20		
		135		0.12		
Sodium Nitrate	4	340	265	0.65	0.40	160.0
		270		0.45		
		250		0.38		
		200		0.25		
Urea	5	345	231	0.60	0.30	156.0
		370		0.70		
		165		0.28		
		160		0.16		
		145		0.20		
Peptone	4	375	345	1.00	0.96	384.0
		350		0.93		
		330		0.70		
		325		1.20		
Guanin	6	400	311	1.07	0.73	292.0
		335		1.04		
		320		0.47		
		275		0.60		
		270		0.70		
		270		0.50		
Guanidin Carbonate..	4	210	171	0.44	0.31	124.0
		175		0.30		
		170		0.25		
		130		0.25		
Benzamid*						
Caffein	6	210	130	0.34	0.23	92.0
		170		0.23		
		140		0.22		
		115		0.22		
		80		0.20		
		55		0.15		
Alanin	6	430	300	1.20	0.88	352.0
		330		1.50		
		330		1.05		
		270		0.55		
		255		0.69		
		185		0.32		
Ammonium Sulfate ..	5	430	340	1.52	0.85	348.0
		345		0.97		
		325		0.75		
		300		0.65		
		300		0.54		
Asparagin	5	420	336	0.90	0.83	332.0
		400		1.34		
		300		0.69		
		285		0.68		
		275		0.57		
Glycocoll	5	325	240	0.82	0.56	224.0
		225		0.40		
		220		0.67		
		220		0.53		
		200		0.37		

TABLE XI—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Uric Acid	5	415	230	1.02	0.48	192.0
		320		0.50		
		215		0.32		
		170		0.29		
		170		0.27		
Diphenylamin*						
Guanidin Nitrate	3	150	135	0.26	0.22	88.0
		130		0.22		
		125		0.20		

* No growth, died within three days.

eral sources of error of which the most serious is the individual differences which occur between plants. No two individuals are exactly alike, as is shown, for instance, by the different growth vigor of different plants under identical external conditions. The degree of this error diminishes with increase in the number of plants. A second factor is the light relation. In the climate of southern Michigan, during the winter months on

TABLE XII
SERIES VI, DENT CORN, STERILE CULTURES
(November, 1915—March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	330	289	1.25	1.07	100.0
		320		1.52		
		300		1.22		
		300		0.90		
		285		1.00		
		220		0.55		
Hemoglobin	5	335	283	2.20	1.02	95.3
		335		0.55		
		280		1.34		
		255		0.60		
		210		0.40		
Casein	4	400	284	3.00	1.46	130.4
		255		1.00		
		250		0.50		
		230		1.35		
Linseed Meal	5	355	313	2.26	1.46	136.4
		330		1.70		
		315		1.05		
		285		1.30		
		280		1.00		
Cottonseed Meal	5	405	338	1.34	1.68	157.0
		370		2.20		
		350		1.65		
		310		1.67		
		255		1.55		
Malt	6	390	328	1.25	1.13	105.6
		380		0.95		
		335		2.35		
		300		0.85		
		300		0.75		
		265		0.65		

account of the shorter days, less intensity of the sunlight and the large proportion of the cloudy days, the light at the disposal of the plants is much less than during the summer months. As a result, the rate of growth is less than in summer. This fact must be taken into consideration when comparing the growth of series which were grown at different times of the year. Also, those plants which were grown in the laboratory windows did not receive as much light as those in the greenhouse, and those standing near the windows received more than plants farther back. This

TABLE XIII

SERIES VI, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(November, 1915—March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	5	360	316	1.65	1.22	100.0
		335		1.15		
		325		1.40		
		310		0.70		
		255		1.40		
Hemoglobin	4	350	264	0.60	0.78	62.3
		260		1.30		
		260		0.85		
		185		0.35		
Casein	3	400	303	2.70	1.54	126.2
		300		1.42		
		210		0.50		
Linseed Meal	4	370	332	2.82	1.61	123.7
		365		1.30		
		315		0.90		
		280		2.19		
Cottonseed Meal	6	365	290	2.98	1.61	123.7
		310		1.50		
		300		1.70		
		280		1.17		
		255		1.25		
		230		1.05		
Malt	5	355	310	0.90	1.01	82.7
		345		1.45		
		340		1.25		
		320		0.90		
		235		0.56		

was controlled by shifting their positions during the period of growth. The diminishing of light causes a lessening of carbohydrate production and hence slower growth. This slow development may somewhat influence the assimilation of nitrogen. Another possible source of error is the wide temperature variations which occurred while some of the series were being grown. During vacations the heat in the building where the plants were grown was reduced and this caused a check in growth in Series V and VI from which the plants did not fully recover. These factors, then, which influence the percentage of error must be born in mind when making comparisons between different series. The large error due to differences in individual plants is well illustrated in the check solution of Series

TABLE XIV

SERIES VII, DENT CORN, STERILE CULTURES
(January-March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Distilled Water	3	275	220	0.93	0.62	47.3
		215		0.55		
		170		0.40		
Check	6	375	324	1.65	1.31	100.0
		360		1.20		
		345		1.30		
		330		1.20		
		305		1.41		
		230		1.15		
Sodium Nitrate	4	400	370	2.75	2.17	185.6
		385		2.60		
		345		1.90		
		290		1.42		
Urea	5	465	375	3.12	2.00	152.6
		430		2.40		
		390		1.73		
		320		1.50		
		270		1.27		
Peptone	5	435	353	2.47	1.78	135.8
		365		1.47		
		350		2.60		
		310		1.05		
		305		1.30		
Guanin	6	400	348	2.15	1.60	122.1
		360		1.90		
		360		1.90		
		350		1.20		
		345		1.42		
		275		1.05		
Alanin	4	335	291	2.00	1.50	114.5
		300		2.45		
		280		0.90		
		250		0.67		
Ammonium Sulfate...	6	495	425	2.70	2.45	187.0
		485		3.85		
		460		3.30		
		400		2.02		
		360		1.70		
		350		1.12		
Asparagin	6	560	523	5.10	3.89	296.9
		550		4.25		
		550		4.20		
		510		2.77		
		500		4.10		
		470		2.97		
Uric Acid	4	560	478	3.85	3.16	241.2
		520		3.35		
		435		2.47		
		400		2.98		
Hemoglobin	6	485	364	3.75	1.92	146.5
		425		2.60		
		365		1.42		
		325		1.20		
		300		1.52		
		285		1.02		

TABLE XIV—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Casein	6	490	422	3.65	2.68	204.5
		460		2.54		
		440		2.80		
		420		3.00		
		400		2.70		
		320		1.25		
Linseed Meal	5	415	327	2.75	1.57	112.2
		335		1.35		
		310		0.97		
		300		1.30		
		275		1.50		
Cottonseed Meal	6	410	321	2.90	1.83	139.6
		400		2.15		
		360		2.10		
		280		1.40		
		245		1.07		
		230		1.37		
Malt	6	330	301	1.25	1.05	80.1
		325		1.25		
		320		1.47		
		310		0.97		
		275		0.77		
		250		0.65		
Creatin	6	400	347	2.20	1.58	120.6
		360		1.45		
		355		1.65		
		350		1.40		
		345		1.45		
		275		1.37		

II. The largest plant measured 365 cm. and the smallest 155 cm., a difference of 210 cm. However, in all the checks of all the series, 48 plants in sterile cultures averaged 227.7 cm., and 49 plants in inoculated cultures averaged 221.6 cm. a difference of only 6.1 cm. With this number of plants the margin of error is very small.

The means for determining the amount of development of the plants in the various compounds used was, as has been stated above, by measurement of the length of the stalks and leaves, and by determining the dry weight. A comparison of the data obtained by the two methods shows that they are nearly parallel. The data show that in 19 cases the measurements and weights are, respectively, in the same relation in the sterile and the inoculated cultures; but in 5 cases they are reversed. This may be partly explained by the fact that the cultures in which these reverses occurred were checked in their growth, as has been explained. The leaves then did not develop well, but ears were formed which increased the weight. The weights probably serve a more definite and accurate basis for comparison than the measurements (cf. Tables X-XVII).

Since the problem was to determine the availability of various organic nitrogenous compounds for higher plants, the most logical means of discussion seems to be to take up each compound separately, explain the re-

TABLE XV

SERIES VII, DENT CORN, INOCULATED WITH *B. SUBTILIS*
(January-March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Distilled Water	2	220	180	0.72	0.50	34.5
		140		0.28		
Check	5	350	297	1.79	1.45	100.0
		330		1.68		
		325		1.65		
		265		1.20		
		215		0.95		
Sodium Nitrate	4	410	350	2.32	1.65	113.8
		370		2.80		
		320		0.80		
		300		0.70		
Urea	5	480	350	2.35	2.10	144.8
		465		3.95		
		405		1.67		
		355		1.65		
		225		0.70		
Peptone	6	490	435	3.45	2.35	162.0
		440		2.32		
		440		1.57		
		430		3.00		
		430		1.70		
		380		2.10		
Guanin	6	495	454	3.55	2.52	173.8
		490		3.10		
		470		2.25		
		465		2.35		
		420		1.92		
		385		1.97		
Alanin	6	400	333	2.97	2.10	144 8
		400		2.95		
		315		2.60		
		305		1.30		
		300		1.40		
		280		1.38		
Ammonium Sulfate...	6	510	462	3.45	3.15	217.2
		465		3.57		
		465		3.17		
		460		2.17		
		445		3.15		
		430		3.41		
Asparagin	4	610	561	4.69	4.24	292.4
		600		4.55		
		535		3.77		
		500		3.95		
Uric Acid	6	480	417	3.32	2.51	173.1
		475		2.52		
		460		3.55		
		400		3.12		
		390		1.60		
		300		0.97		
Hemoglobin	6	550	502	4.30	3.61	248.0
		540		3.15		
		535		4.27		
		510		3.80		
		505		3.65		
		375		2.49		

TABLE XV—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Casein	4	485	464	4.85	3.79	261.4
		470		3.42		
		450		3.57		
		450		3.35		
Linseed Meal	6	425	360	3.10	2.08	143.4
		375		1.65		
		350		1.95		
		350		1.32		
		330		2.85		
		330		1.60		
Cottonseed Meal	6	460	380	3.20	2.59	178.6
		400		2.82		
		370		2.20		
		370		2.12		
		360		2.52		
		315		2.70		
Malt	6	380	331	1.68	1.50	103.4
		375		1.55		
		370		1.75		
		360		1.92		
		270		1.10		
		230		1.02		
Creatin	6	390	340	1.60	1.44	99.3
		360		1.45		
		340		1.20		
		325		1.72		
		320		1.17		
		300		1.50		

sults obtained in the different cultures, and show the significance which they seem to reveal. Therefore, this procedure has been adopted.

Checks

The check solution was used in all the series. It contained all the chemical elements necessary for the growth of plants except nitrogen and those which the plant gets from the air. The growth in this solution was taken as the amount of growth allowed by the nitrogen supply in the seed. In the culture solutions containing nitrogen a growth markedly less than that of the check was interpreted as a toxic effect. A growth equal to that of the check was assumed to indicate that the nitrogen was not available. A growth markedly better than the check indicated that nitrogen in the form supplied was available. The plants grown in the check solution, toward the end of the period of growth, always showed the yellowing of the leaves, a characteristic effect of the lack of nitrogen.

The difference between the plants grown in the sterile cultures and in the inoculated ones is very slight. With the pop corn the plants inoculated in all cases were from 10 to 40 cm. better. Considering the measurement on length of all the check plants, those in the sterile cultures averaged 6 cm. per plant better than those in the inoculated.

Sodium Nitrate

A complete nutrient solution containing sodium nitrate was employed in all the series but one. Since the time of Boussingault (8) sodium nitrate has been considered one of the best, if not the best, source of nitrogen. It is in common use as a commercial fertilizer. In Series II, III, and VIII of the experiments it produced the best growth of all substances used. These were all grown in the greenhouse under favorable

TABLE XVI

SERIES VIII, DENT CORN, STERILE CULTURES

(February-April, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	170	157.5	1.52	1.23	100.0
		170		1.25		
		160		1.10		
		155		1.30		
		150		1.25		
		140		1.00		
Sodium Nitrate	5	215	204.0	1.90	1.76	143.0
		215		1.80		
		205		1.72		
		200		1.60		
		185		1.80		
Urea	3	190	181.6	1.85	1.68	136.5
		190		1.70		
		165		1.50		
Uric Acid	6	200	179.0	1.80	1.50	126.8
		190		1.70		
		190		1.50		
		185		1.50		
		160		1.40		
		150		1.45		
Casein	6	205	185.0	1.65	1.41	114.6
		200		1.45		
		190		1.70		
		175		1.25		
		170		1.25		
		170		1.20		
Cottonseed Meal	5	160	148.0	1.28	1.14	92.6
		155		1.20		
		145		1.10		
		145		1.10		
		135		1.02		

conditions of temperature and light. In Series VIII, the experiment was discontinued after only two months had elapsed because with the small amount of medium used the water was exhausted at the end of that period.

Table VII and figure 1 show that in the growth of pop corn, sodium nitrate in sterile cultures was the best of the compounds tested as a source of nitrogen, while in the inoculated cultures urea equaled it in value. In the growth of dent corn the results in sterile cultures indicated that ammonium sulfate and asparagin are superior to sodium nitrate as a source

of nitrogen. In inoculated cultures, however, the following substances gave better results than the nitrate: asparagin, ammonium sulfate, peptone, guanin, uric acid, alanin, urea, hemoglobin, casein, linseed and cottonseed meals. The growth of the dent corn plants in the inoculated cultures of the nitrate was slightly poorer than in the sterile cultures, while

TABLE XVII

SERIES VIII, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(February-April, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	155	137.5	1.20	1.03	100.0
		155		1.15		
		140		1.15		
		130		0.80		
		125		0.92		
		120		0.95		
Sodium Nitrate	6	205	195.0	2.15	1.88	182.5
		205		1.82		
		205		1.85		
		195		1.80		
		190		2.10		
		170		1.55		
Urea	6	180	166.0	1.75	1.61	156.3
		175		1.88		
		175		1.35		
		170		1.90		
		160		1.90		
		135		0.90		
Uric Acid	5	190	174.0	1.80	1.57	152.4
		190		1.60		
		180		1.50		
		170		1.00		
		140		1.95		
Casein	6	215	185.0	1.92	1.56	151.4
		195		1.47		
		190		1.73		
		185		1.67		
		165		1.27		
		160		1.32		
Cottonseed Meal	6	180	161.0	1.45	1.18	114.5
		180		1.25		
		160		1.30		
		155		1.25		
		155		1.10		
		135		0.72		

in the pop corn plants the reverse was true, but the differences in both cases were within the range of error inherent in the method.

From these experiments it is clear that in all cases the growth of plants when furnished sodium nitrate was markedly better than when no nitrogen, except that in the seed, was present. The poorest showing for the nitrate was 113.6 per cent of the check in Table XV; the best was 236.6 per cent in Table X.

Urea

The plants grown in cultures containing urea as the source of nitrogen showed in the case of the pop corn a decidedly better development than those in the check solution. This indicates that the nitrogen of urea is available to some extent, but not sufficiently to make urea equal to sodium nitrate. However, in the inoculated cultures it proved equal to the nitrate as a source of nitrogen. This indicates that ammonification or some other transformation of urea is necessary for the best utilization and assimilation of that compound by pop corn plants. The weight of the dent corn plants in the sterile cultures showed urea to be about 50 per cent better than the check, though the leaf measurements were no greater

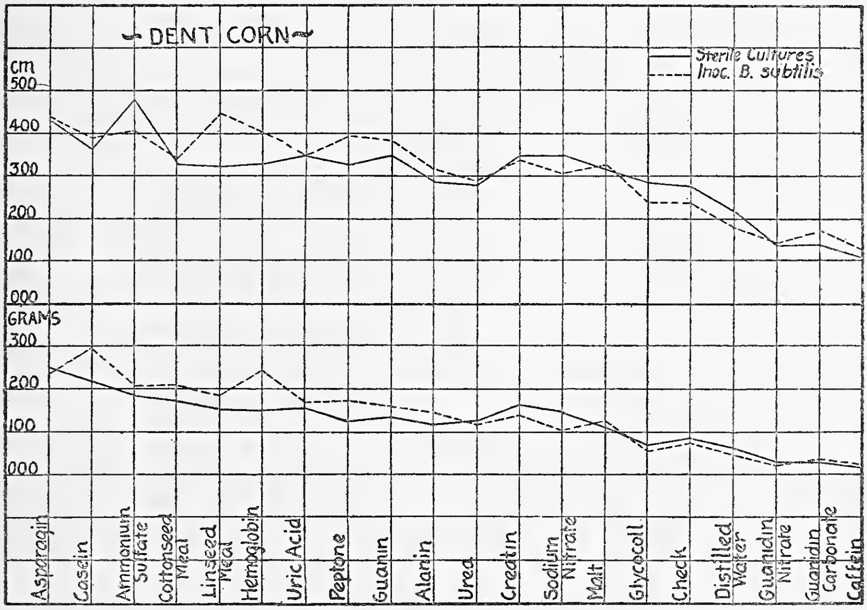

Fig. 2.—A summary of all the dent corn plants grown, showing both the dry weights and the measurements.

than those of the check. In the inoculated cultures both length and dry weight showed urea better than the inoculated check, and the dry weight showed even better growth than the dry weight of the plants in the inoculated cultures of sodium nitrate. A comparison of the sterile and the inoculated cultures, containing urea, shows no difference in the dry weight and the length of leaf is only slightly better in the inoculated cultures.

Urea was found unavailable by Pryanishnikov and Lyebyedyev (40), and toxic by Sawa (41). However, it has been reported beneficial by Hutchinson and Miller (16), Molliard (33), Suzuki (52) and Tompson (54). Takeuchi (53) has found that the enzyme, urease, which ammonifies urea, is not present in *Mays*. The corn itself then cannot ammonify urea.

Peptone

Peptone was utilized by *Mays* plants in both sterile and inoculated cultures. In the sterile cultures in both varieties of corn it was better than urea but did not quite equal the nitrate as a source of nitrogen. As with urea, the action of *B. subtilis* seemed to increase its availability for *Mays* plants. With pop corn, in the inoculated cultures, the growth was not equal to either that in the nitrate or urea but was considerably better than in the sterile cultures. With dent corn in sterile cultures the plants make a much better development than in the check but were not equal to those in the nitrate. The growth was 27 per cent better in the inoculated cultures than in the sterile. The plants with peptone were fifth in rank. Hutchinson and Miller (16) have found peptone a source of available nitrogen.

Guanin

Guanin was found to be exceedingly toxic to the pop corn plants in both sterile cultures and in those cultures which were inoculated with *B. subtilis*. These plants made practically no growth. In the cultures inoculated with soil water the growth was fair, but as there were only a few plants, no definite conclusions can be drawn. In the sterile cultures it was found to be about equal to the sodium nitrate for dent corn, and was better in the inoculated than in the sterile cultures.

The results show clearly that as a source of nitrogen the same chemical compound may have a value differing to a considerable degree for different varieties of a species. Guanin was toxic to pop corn and furnished available nitrogen to dent corn. Schreiner and Reed (44) and Schreiner and Skinner (46) have found guanin available to wheat seedlings.

Guanidin Carbonate

In the experiments guanidin carbonate was found to be exceedingly toxic to the pop corn plants used. Young seedlings made a very slight growth and died within a few days on culture media containing this substance. It was less toxic to dent corn but none of the cultures with this substance were as good as the check. Guanidin carbonate has also been found quite toxic to other plants by Kawakita (18), Schreiner and Reed (44, 45), Schreiner and Skinner (46), and Bierma (4).

Benzamid

The plants grown in a nutrient solution containing benzamid showed a decidedly toxic effect of this substance. They made a very feeble growth and died within 3 weeks. The action of *B. subtilis* did not alter the toxicity of this substance. Lutz (30) found that all compounds containing the benzin ring group were toxic to plants.

Caffein

The caffein nutrient solution with dent corn was more toxic than the guanidin carbonate, and much poorer than the solution used in the check culture. The leaves of the plant were small and pale in color. Those plants grown in the inoculated cultures were slightly better than those in the sterile cultures but the difference was not very marked. This compound has also been reported toxic by Lutz (30).

Glycocoll

Glycocoll was used as the source of nitrogen in only one series of experiments. The results of this series showed that it was favorable to the growth of *Mays* plants. It did not prove equal to sodium nitrate in the single series in which it was used. The growth was very little better in the sterile cultures. Its effect on other plants has been ascertained and found favorable by Schreiner and Skinner (46), Hutchinson and Miller (16), Dachnowski and Gormley (12), Lefevre (24), Molliard (34), and Schreiner and Reed (47).

Uric Acid

Thompson (54) has shown by his experiments that uric acid furnishes as good a source of nitrogen for oats as does urea and sodium nitrate. The results of the author's experiments with *Mays* are very similar. In the sterile cultures the growth of the dent corn was equal to that in the sodium nitrate both in length of leaves and in dry weight. Uric acid was better than urea as a source of nitrogen. There was only 1 cm. difference by measurement and .05 gm. by weight, between the averages of the sterile and the inoculated cultures in uric acid.

Diphenylamin

Diphenylamin was the most toxic substance used. When germinated seedlings were placed upon the agar medium containing this substance the roots turned brown and the plants died within 24 hours.

Alanin

The results of the experiments with alanin as a source of nitrogen, presented in Tables X, XI, XIV and XV, show it to be a good nitrogen source. In the sterile cultures the plants are nearly as good as those in the corresponding nitrate solution. The plants grown in the inoculated nitrate cultures are better than those of the sterile and better than the inoculated nitrate cultures. It is, therefore, a good source of nitrogen for *Mays*, although Schreiner and Skinner (46) found it slightly toxic to wheat seedlings and Molliard (34) reported it toxic to roots, while Lefevre (24) found it favorable as a nitrogen source.

Ammonium Sulfate

Ammonium sulfate was used in Series V and VII of the experiments. It has been known from the time of Liebig to be very readily assimilated by some plants. In the experiments here reported it was found to give a better growth of *Mays* than most of the other substances tried, and much better than sodium nitrate or urea. The measurements of the leaves show the sterile cultures to be slightly better while the weights reverse the ratio.

Asparagin

Asparagin is a substance found very widely distributed in plants, and the results obtained in these investigations show it to be an excellent source of nitrogen for *Mays*. The plants grown in this solution in sterile cultures are shown by measurements to be surpassed only by those in ammonium sulfate; by weights they are far better than any others. The growth in inoculated cultures is about equal to that in sterile. It has also been found readily assimilated in the experiments of Baessler (2), Molliard (33, 34), Nakamura (37), and Skinner and Beattie (51).

Guanidin Nitrate

The effect of guanidin nitrate upon *Mays* was about parallel to that of guanidin carbonate; approximately the same growth was obtained, the former showing about the same toxic reaction as the latter. There was a difference in weight of only .02 gm. between the plants in the sterile cultures inoculated with *B. subtilis*.

Hemoglobin

Hemoglobin is a complex animal protein, and it might be expected that, due to the molecular structure, the nitrogen would not be available for plants. The results show that in the sterile cultures it was slightly better than the check both by measurements and weights, but not as good as sodium nitrate. However, in the inoculated cultures the growth was about 25 per cent better than in the sterile, and much better than the inoculated check and nitrate cultures. The plants in this culture were among the best of all the cultures. A part of these plants supplied with hemoglobin were grown in very poor light and this may have had some detrimental influence, but even under such conditions they did exceptionally well.

Casein

Casein, like hemoglobin, is an animal protein, and might be thought to be unavailable for plant nutrition. Kelly (20) found that it may be readily ammonified by soil bacteria. The author's experiments show that in the sterile cultures it is favorable, about equal to sodium nitrate, and that in the inoculated cultures it is considerably better than the nitrate as a source of nitrogen. The inoculated cultures made a greater development than the sterile.

Linseed Meal

That such products as linseed meal and cottonseed meal might be used as a source of nitrogen was suggested by Kelly (19). The results here reported show that plants furnished with linseed meal make a slightly better growth in the sterile cultures than the check plants, but not equal to that of the plants having sodium nitrate. The inoculated cultures with linseed meal were decidedly better than the sterile and also better than the inoculated nitrate cultures.

Cottonseed Meal

The results with cottonseed meal were very similar to those with linseed meal. That is, in the sterile cultures the growth was only slightly better than the check but in the inoculated it is markedly better, and the plants here were among the best of all the cultures.

Malt

The plants grown in the solution to which malt had been added made approximately the same growth as those in the check in both the sterile and the inoculated cultures. This substance furnished practically no nitrogen, nor did the bacteria have any influence on the availability of the inoculated nitrate cultures.

Creatin

Creatin was used only in Series VII. This compound was of some value as a source of nitrogen, as indicated by the growth, which was somewhat better both in the sterile and in the inoculated cultures than the respective check cultures. There was little difference between them in growth in the sterile and in the inoculated cultures when the creatin was used as the source of nitrogen.

Chemical Groups

The inorganic nutrient salts, sodium nitrate and ammonium sulfate were both highly beneficial to plant growth as has already been stated, but they were excelled by some of the organic compounds. Among the organic compounds used were three purin derivatives. One, uric acid, was found available and decidedly beneficial, another guanin, was also found favorable to dent corn, while the third, caffein, containing three methyl groups, was quite toxic. The amids of the simple organic compounds are shown to contain nitrogen available for plant growth. Glycocoll and alanin are amids of acetic and propionic acids, respectively. Asparagin is a monamid of amido succinic acid and was one of the most favorable substances experimented with. Urea might be considered a diamido-carbonic acid, the simplest of all the organic acids. The albuminoid substances peptone, casein and hemoglobin were also available for plant nutrition.

The guanidin derivitives, guanidin carbonate, guanidin nitrate and creatin appeared to furnish the plant with no nitrogen. The first two were noticeably toxic in their action, while creatin seemed free from toxic properties.

Two compounds of the benzin ring group were used, benzamid and diphenylamin, the former with one, the latter with two benzin rings in the molecule. Both of these compounds were highly toxic to the *Mays* plants. The results with these two compounds are in accord with the work of Lutz (30) who has reported that benzylamin, diphenylamin, analin, and naphthylamin, members of the benzin series, were all toxic to the plants he employed.

Of the ground seeds, cottonseed meal and linseed meal contained available nitrogen for the plants, while malt was of no value as a source of nitrogen.

The results of this work and that of other investigators lead us to believe that some substances containing organic nitrogen may be used as a source of this element for plants in general. The fact that plants under experiment can absorb some of the substances, without first being broken down, indicates that this can take place with the plants in the fields since they grow in soils containing manure or other decaying vegetable and animal matter. Some of the substances then, in fertilizers, are directly assimilable by the plants and do not need to be ammonified and nitrified as is usually thought. Also, products probably occur in the intermediate stages of decomposition that may be directly utilized by plants. This is contrary to the general belief in agricultural practice that plants must be furnished with either ammonium compounds or nitrates. Nevertheless, most of the substances tried were utilized better or more rapidly when acted upon by *B. subtilis*. This is intelligible if *B. subtilis* causes ammonification of such substances, since ammonium sulfate was better than sodium nitrate.

Conclusions

The results of the investigations reported in this thesis warrant the following conclusions:

1. *Zea Mays* directly assimilates and uses the following organic nitrogenous compounds named in the order of their availability, asparagin, casein, cottonseed meal, hemoglobin, linseed meal, uric acid, peptone, guanin, alanin, urea, creatin, malt and glycocoll.

2. The following organic nitrogenous compounds are toxic to the growth of *Zea Mays:* guanidin carbonate, guanidin nitrate, diphenylamin, caffein, and benzamid. Guanin is toxic to pop corn but not to dent corn.

3. Eight organic substances which were directly available produced better growth when acted upon by *B. subtilis,* probably because of ammonification. These were peptone, guanin, alanin, linseed meal, cotton-

seed meal, casein, hemoglobin and urea. The last showed this effect only with pop corn.

4. The availability of the following substances was not increased by the action of *B. subtilis:* urea in the case of the dent corn, sodium nitrate, asparagin, ammonium sulfate, uric acid, malt, creatin, glycocoll, and those compounds which were toxic.

5. In the case of dent corn 6 substances were better than sodium nitrate; cottonseed meal, linseed meal, casein, hamoglobin, uric acid, and asparagin. The following, though available, were not better than sodium nitrate: urea, peptone, guanin, alanin, and creatin.

6. The different varieties of the same species of corn react differently with some nutrient substances. Guanin was toxic to pop corn but available to dent corn. Peptone was better utilized by dent corn than by pop corn.

7. The compounds of the benzin ring were found exceedingly toxic to the plants tried.

8. Ammonium sulfate is a far better source of nitrogen for dent corn than sodium nitrate, and is surpassed only by casein and asparagin, when tested by the dry weight, and only by asparagin when tested by length of leaves produced.

9. Generally, those organic compounds of high complexity in composition are better after ammonification, while those of a low degree of complexity are not improved by ammonification.

10. Very likely nitrification following ammonification would be detrimental, since sodium nitrate was not equal to ammonium sulfate for dent corn.

11. The method of measuring growth by length of leaves gave results very nearly parallel to those obtained by determining the dry weight, and is much simpler.

These conclusions apply to the two varieties of corn plants used. Only experiments on other species and varieties will show how they react to these substances.

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PLATE I

Fig. 1.—One of the 1000-c.c. culture flasks used in growing the corn plants; show- the rolled cotton plug and through it passing the glass tube, in which the plant grew through the cotton plug.

Fig. 2.—The 168 dent corn plants of Series VII. The flasks to the left of each number were sterile and to the right inoculated with *B. subtilis.* The various compounds used are : O, distilled water; I, check; II, sodium nitrate; III, urea; IV, peptone; V, guanin; IX, alanin; X, ammonium sulfate; XI, asparagin; XIII, uric acid; XVI, hemoglobin; XVII, casein; XVIII, linseed meal; XIX, cottonseed meal; XX, malt; XXI, creatin.

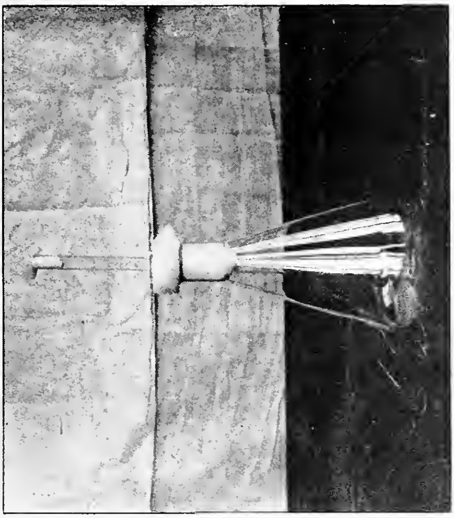

Fig. 1

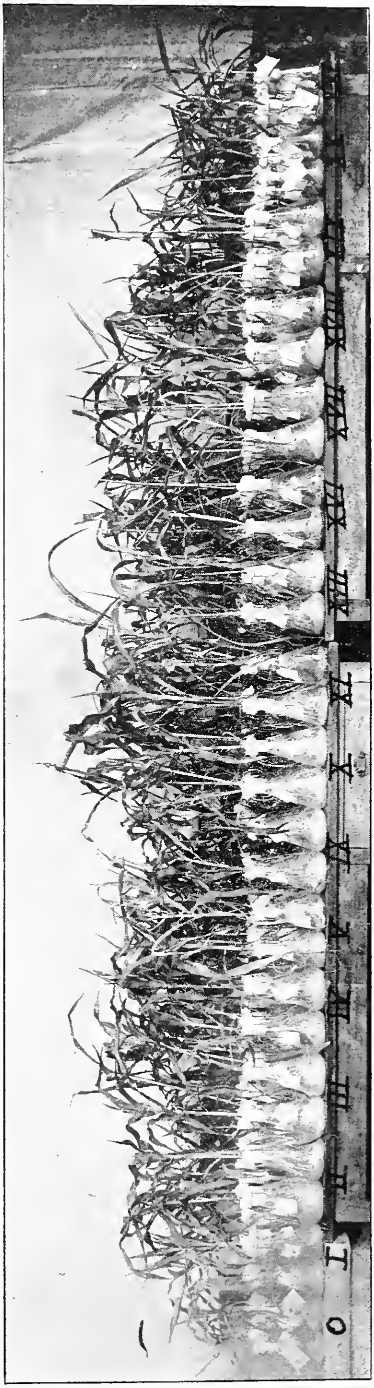

Fig. 2

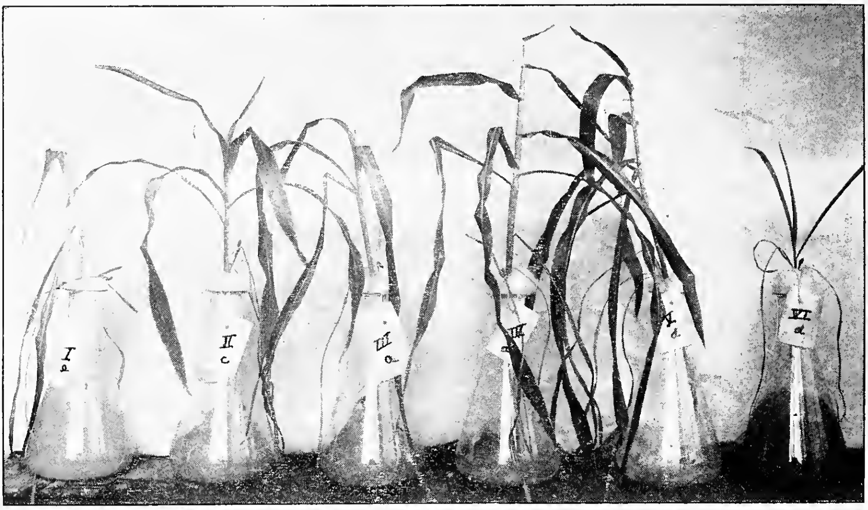

Fig. 1

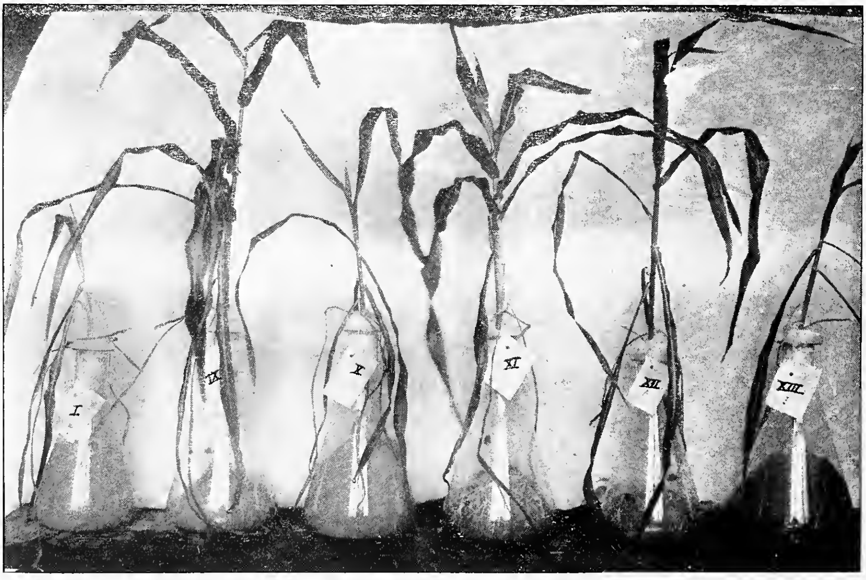

Fig. 2

PLATE II

Fig. 1.—Dent corn plants grown under sterile conditions of Series V, with different forms of nitrogen added to the stock solution, namely, I, check; II, sodium nitrate; III, urea; IV, peptone; V, guanin; VI, guanidin carbonate.

Fig. 2.—Continuation of figure 1: IX, alanin; X, ammonium sulfate; XI, asparagin; XII, glycocoll; XIII, uric acid.